Influence of thyroid hormone on nicotinamide metabolism in the rat

The thyroid hormone is known to influence many metabolic processes in the body. From the recent work of LARDY and co-workers[1,2] and of HOCH AND LIPMANN[3] on the effect of thyroxine on oxidative phosphorylation, it can be inferred that synthetic processes that are endergonic and so in general dependent on high energy phosphate for their completion will be inhibited in the thyrotoxic state. But experimental evidence obtained in a number of cases of anabolic processes such as synthesis of fatty acid[4], cholesterol[5], liver protein[6] and coenzyme A[7] is not in conformity with this concept. In fact this confusing state of knowledge is well brought out when the effect of the thyroid hormone on methylation reactions is studied. Thus ASTRUP AND STEEWSHOLT[8] observe that the thyroid gland has no role in the methylation of glycocyamin, whereas CALVO *et al.*[9] working on the methylation of nicotinic acid conclude that the thyroid hormone stimulates transmethylation. In the light of the demonstrated uncoupling of phosphorylation from respiration in thyrotoxicosis[1-3] and of the recent observation that high energy phosphate is essential for methylation of nicotinamide[10], the metabolic changes undergone by nicotinamide under conditions of thyroid imbalance were studied and the results are reported here.

Twelve albino rats weighing 125–130 g were divided into three groups of four each. The first group served as control, the second was rendered hypothyroid by giving methylthiouracil with the stock diet and the third group was given desiccated thyroid along with the stock diet to render it hyperthyroid. Five to seven weeks were required for the rats to reach the hyperthyroid and hypothyroid states. The rats were then given each a dose of 2.5 mg of nicotinamide intraperitoneally, transferred to individual metabolism cages and urine was collected for the following 48 hours in 3 ml of acetic acid under toluene. The tertiary derivatives of nicotinic acid in the urine samples were determined microbiologically using *Lactobacillus arabinosus* 17–5 as the assay organism[11] and N'-methylnicotinamide was estimated by the fluorimetric method of CARPENTER AND KODICEK[12].

In Table I are presented the results showing the excretion of N'-methylnicotinamide under normal, hypo- and hyperthyroidal conditions, expressed as per cent total nicotinic acid derivatives excreted.

TABLE I

EXCRETION OF N'-METHYLNICOTINAMIDE (NMN) BY CONTROL, HYPO- AND HYPERTHYROIDAL RATS

Experimental condition of rats	*NMD excreted as per cent total nicotinic acid derivatives* excreted*		*Per cent deviation from normal*
	Range	*Mean*	
Normal	47–60	54.0	—
Hypothyroidal	71–85	78.0	+46.0
Hyperthyroidal	28–50	39.0	—27.0

* Total nicotinic acid derivatives are made up of the tertiary derivatives of nicotinic acid assayed by *L. arabinosus* and NMN estimated by the fluorimetric method.

The decreased excretion of N'-methylnicotinamide by the hyperthyroid rat could be due to more than one reason. Thus it has been pointed out earlier[10] that adenosine triphosphate (ATP) is essential for the overall process of transmethylation as an activating agent of methionine, the methyl group donor. If it is accepted that the mechanism of action of the thyroid hormone is the uncoupling of phosphorylation from respiration[1-3], it should follow as a corollary that the generation of high energy phosphate (ATP) *in vivo* and therefore ATP-dependent metabolic reactions would be impaired in the thyrotoxic state of the animal; in this way the formation of N'-methylnicotinamide could be expected to be decreased in the hyperthyroid condition. It is interesting in this connection that another high energy phosphate-dependent reaction, namely the conjugation of benzoic acid with glucuronic acid, has been found to be decreased in hyperthyroid rats[13].

The second circumstance which might be responsible for the lowered excretion of N'-methylnicotinamide by hyperthyroid rats is an inducement of vitamin B_{12} deficiency in the hyperthyroid state. Vitamin B_{12} is known to be capable of relieving thyrotoxic symptoms[14] and thyroxine in the form of iodocasein has been used for producing vitamin B_{12} deficiency in experimental animals[15]; it is, however, possible that the result of such an administration of the thyroid hormone may be more drastic than the supervention of a simple deficiency of vitamin B_{12}. Further, it has been demonstrated that vitamin B_{12} may be involved in many reactions featuring the "single carbon unit" including methylation processes[16]. Thus the decreased excretion of N'-methylnicotinamide by hyperthyroid rats may also be a reflection of vitamin B_{12} deficiency setting in during the hyperthyroid state.

The increase in the excretion of N'-methylnicotinamide by methylthiouracil-treated rats, which is even more pronounced than the decrease observed in the case of the hyperthyroid animals, is, therefore, to be expected since it represents an effect opposite to that produced in hyperthyroidism. However, on the basis of the incomplete present day knowledge of the mechanism of action of the thyroid hormone in the normal living system, no precise explanation of this pronounced enhancement of methylation in hypothyroidism can be given. It may, however, be mentioned that a similar influence of thyroid imbalance on the acetylation of sulphanilamide has been observed by FRAENKEL-CONRAT AND GREENBERG[17] and on the synthesis of acetylcholine by GUZMAN[18].

The decreased methylation of nicotinamide in hyperthyroidism, observed in the present study, does not, therefore, favour the postulate of CALVO *et al.*[10] that the thyroid hormone stimulates transmethylation.

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Occurrence of free nucleotides in *Penicillium chrysogenum*

In the course of studies on the metabolism of *Penicillium chrysogenum* it was thought of some importance to investigate the nature of the nucleotides occurring in the mycelium of this organism. Only adenosine-5'-monophosphate and adenosine-5'-triphosphate have hitherto been identified in *P. chrysogenum*[1].

The mycelium used in this study (strain Wis 49–133) was grown in a stirred fermenter in the synthetic medium of JARVIS AND JOHNSON[2], containing 22.5 g lactose, 7.5 g glucose, 3 g ammonium acetate and 5 g ammonium lactate, per liter. The mycelium was removed from the fermentation after 50 hours when the reducing sugar content was approximately 0.5%, filtered by suction, washed with water and kept at −20° C. The nucleotides were extracted with 50% ethanol and precipitated with mercuric acetate, as described by CAPUTTO, LELOIR, CARDINI AND PALADINI[3]. The supernatant obtained after decomposition of the mercury salts, with hydrogen sulphide was fractionated by chromatography on Dowex-1 formate with the formic acid system of HURLBERT, SCHMITZ, BRUMM AND POTTER[4]. Eleven peaks were detected by measuring the optical density of the fractions at 260 mμ. The fractions comprising each peak were pooled and lyophilized, either directly or after adsorption and elution on charcoal[5], and then further fractionated by large-scale paper chromatography on Whatman No. 1 or 3 MM using ethanol-ammonium acetate (pH 3.8) as solvent[6]. Each ultraviolet-absorbing band was cut out, washed with ethanol, and then eluted with water at 5° C. The solutions thus obtained were used to identify the compounds on the basis of the following criteria: (a) type of U.V. spectrum in acid, alkaline and neutral solution, (b) mobility on paper with four different solvents in parallel with corresponding authentic nucleotides, (c) type of U.V. spectrum and mobility on paper (using two different solvents) of the base obtained after hydrolysis with perchloric acid, (d) ratio of base to organic phosphate to ribose (for most of the purine-nucleotides), (e) hydrolysis with 5'-nucleotidase from bull seminal plasma (for monophosphates)[7], (f) type of sugar released on mild acid hydrolysis